High Temperature Gas Chromatography

P. Sandra, University of Ghent, Ghent, Belgium
F. David, Research Institute for Chromatography, Kortrijk, Belgium

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Introduction

Gas chromatography (GC) is generally believed to be restricted to the analysis of 'volatiles' and is less applicable to the analysis of so-called 'heavy' compounds. The introduction of persilylated glass and fused silicea columns, of thermostable stationary phases and of non-discriminative injection devices in capillary GC (CGC) have made the definitions of 'volatile' and 'heavy' very flexible.

High temperature CGC (HTCGC) was developed in the 1980s as a result of work carried out by Grob, Geeraert and Sandra, Trestianu *et al.*, Lipsky and Duffy, and Blum and Aichholz (see Further Reading). Although HTCGC was initially not accepted as a 'robust' analytical technique for the analysis of high molecular weight compounds, in recent years several research groups have demonstrated the capabilities of HTCGC for the analysis of hydrocarbons with carbon numbers in excess of 130 (simulated distillation), of lipid compounds, of emulsifiers, of detergents, of polymer additives, of oligosaccharides, of porphyrins and of many more solutes.

Defining a temperature in HTCGC is not straightforward but it is now generally accepted that 420°C is the maximum allowable column temperature limit in practice. Applications at higher column temperatures have been carried out but, with the exception of hydrocarbons, most organic compounds do not withstand temperatures higher than 420°C. Moreover, the maximum allowable operating temperatures (MAOT) of the stationary phases applied in HTCGC are all lower than 450°C. A prerequisite in HTCGC is that the solutes to be analysed are thermally stable and can be volatilized. The thermal stability of organic compounds depends not only on their nature, but also on the activity of the environment to which they are subjected and on the thermal stress given to the solutes. HTCGC is nowadays performed in a completely inert system, i.e. high purity carrier gas, dedicated and purified stationary phases, fused silica columns with less than 0.1 ppm trace metals and specially deactivated, etc. Moreover, thermal stress is reduced by applying cool on-column (COC) or programmed temperature vaporizing (PTV) injection. Lipids may serve as a good illustration. When oils or fats are used in food preparation, they decompose (formation of volatiles) or polymerize (formation of dimers, trimers, etc.) as a function of time, which makes them no longer useful for cooking. These alterations are caused by the presence of water and oxygen. When heated under inert conditions, however, fats and oils are stable and evaporate. **Figure 1** shows the thermogravimetric profiles for triolein with a molecular mass of 886 Da under a stream of pure nitrogen (A) and of air (B).

In present state-of-the-art HTCGC, the systems are operated under the circumstances shown in curve A. Volatility, on the other hand, is related to the vapour pressure (boiling point) of the compounds. Polydimethylsiloxanes with molecular masses as high as 5000 Da are volatile enough to be analysed by HTCGC, whereas low molecular weight (oligo)saccharides, for example, are not volatile at all. This is because of the polarity of the functional groups. Derivatization is often employed to impart volatility and to yield a thermostable product, thereby also improving chromatographic performance and peak shapes. Silylation, alkylation and acylation are used to modify the active hydrogen in compounds containing –OH, –COOH, and $–NH_2$ functionalities.

HTCGC is a reliable analytical method if a number of prerequisites are fulfilled. The different aspects of the technique are discussed and the potential illustrated with a number of relevant applications.

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Instrumentation for HTCGC

Columns and Stationary Phases

Different support materials have been applied in HTCGC. Leached and deactivated borosilicate glass provides an excellent surface for high temperature

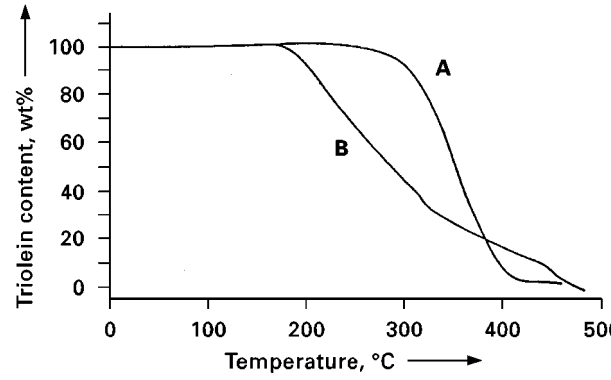

Figure 1 Thermogravimetric analysis of triolein. Ramp from 20 to 500°C at 5°C min^{-1}. (A) pure nitrogen, (B) air.

work. The surface can be coated with different high temperature phases for applications up to 450°C. Glass columns are, however, not really accepted for routine work as they break easily and are difficult to handle. Nevertheless, some laboratories still use glass columns because of their excellent performance for specific applications (Blum and Aichholz). Leached and persilylated fused silica is nowadays mostly applied. The outer polyimide coating of classical fused silica open tubular (FSOT) columns withstands temperatures up to 400°C, thus covering most of the applications of HTCGC. To increase the lifetime of the columns, they are often wrapped in aluminium foil to avoid contact with oxygen which initiates polyimide decomposition. Aluminium-clad fused silica columns have been introduced for applications up to 450°C. Because of the different expansion coefficients of fused silica and aluminium, the columns can become brittle under continuous heating and cooling conditions. An excellent alternative to glass, polyimide-coated fused silica and aluminium-clad fused silica columns are the recently introduced metal columns for which the active surface has been passivated, for example with a thin layer of fused silica. Silcosteel and Ultimetal capillary columns for high temperature work are commercially available. The suppliers both make special columns to perform simulated distillation by GC according to ASTM method D 2887.

A large number of stationary phases have been synthesized for HTCGC. The group of W. Blum has been very active in this respect. Their HTCGC experiments, including the synthesis of the phases and the coating of capillary columns, are summarized in an excellent book. The three phases most often applied in HTCGC are methylsilicone, diphenyldimethylsilicone with phenyl contents varying between 5% and 65% and a carborane-modified methylsilicone. The main reason for this is that high temperature columns with these phases are commercially available. The phases are OH-terminated and immobilization by polycondensation takes place at high temperatures after coating. This increases thermal stability and makes the columns solvent resistant.

Inlet Systems

Different injection devices for CGC have been developed over the years, but only two are applicable in HTCGC, namely COC and PTV injection. With both devices the sample can be introduced at low temperatures avoiding solute alteration or discrimination. For cool on-column injection, an injector device with an elongated secondary cooling tube is advised because this enables high oven temperatures to be used. A hot injector such as a split/splitless injector, has been used to analyse quaternary ammonium salts. At injection temperatures above 360°C the salts are demethylated and the resulting tertiary amines can be analysed by HTCGC. Standard deviations are, however, quite high.

Carrier Gas and Mode of Delivery

Carrier gases with fast diffusion properties, i.e. hydrogen and helium, should be used. When the viscosity is also taken into account, namely $1990 \times 10^{-7}\,\mathrm{g\,cm^{-1}\,s^{-1}}$ for helium versus $840 \times 10^{-7}\,\mathrm{g\,cm^{-1}\,s^{-1}}$ for hydrogen, the performance of the latter is much better because the H vs. u plot at high temperatures is relatively flat whereas the plot for helium is much steeper. If, for safety reasons, hydrogen cylinders are not allowed, a hydrogen generator can offer a solution. The use of electronic pneumatic control (EPC) allows column flow to be maintained constant or even increased during a temperature programmed run, reducing elution times and temperatures. The effective operating range is thereby extended compared to constant pressure operation.

The features of EPC are illustrated with the analysis of some polymer additives (**Table 1**). The sample mixtures include a wide range of additive types, including polar and labile compounds as well as high molecular weight components. Irganox 1010 (compound 24) with molecular mass 1176 Da is particularly important here as an indicator of HTCGC capabilities in polymer analysis. The sample was analysed on a 25 m × 0.32 mm i.d. fused silica open tubular column coated with a 0.17 μm film of methylsilicone. Hydrogen, delivered at a constant pressure (50 kPa) or in the constant flow mode, was the carrier gas. The column temperature was programmed from 80°C to 380°C at 10°C $\mathrm{min^{-1}}$. Cool on-column injection was carried out in the oven track mode, which means that the injector and the column are then programmed at the same rate, and the FID detector was set at 380°C. The analysis shown in **Figure 2** was done under isobaric conditions. The peak shapes and resolution are good but Irganox 1010 could not be eluted at 380°C. For the 25 m × 0.32 mm i.d. column, 50 kPa hydrogen corresponds to 58 $\mathrm{cm\,s^{-1}}$ carrier gas velocity at the initial oven temperature (80°C) but only to 39 $\mathrm{cm\,s^{-1}}$ at the end of the run (380°C). For the chromatogram shown in **Figure 3**, the analysis was carried out in the constant flow mode. In this operating mode, the pressure is automatically increased (from 50 to 112 kPa in this case) as oven temperature increases, to maintain the initial flow rate throughout the run. Under these conditions, Irganox 1010 elutes at 32 min. The use of cool on-column injection with electronic pneumatic control also provides excellent repeatability in retention

Table 1 List of polymer additives

No	*Name*	*Empirical formula*	*MW*	*% RSD on t_R*	*% RSD on peak area*
1	BHA	$C_{11}H_{16}O_2$	180	0.04	0.42
2	Diethylphthalate	$C_{12}H_{14}O_4$	222	0.02	0.29
3	Dibutylphthalate	$C_{16}H_{22}O_4$	278	0.02	0.48
4	Tinuvin P	$C_{13}H_{11}ON_3$	225	0.03	1.48
5	Triphenylphosphate	$C_{18}H_{15}O_4P$	326	0.02	1.77
6	Dicyclohexylphthalate	$C_{20}H_{26}O_4$	330	0.02	0.55
7	Dioctylphthalate	$C_{24}H_{38}O_4$	390	0.02	0.24
8	Tinuvin 327	$C_{20}H_{25}ON_3Cl$	357	0.02	0.42
9	Benzophenone UV 531	$C_{21}H_{26}O_3$	326	0.01	0.98
10	Erucamide	$C_{22}H_{43}ON$	337	0.02	1.74
11	Tinuvin 770	$C_{28}H_{52}O_4N_2$	480	0.01	0.30
12	Irgaphos 168	$C_{42}H_{63}O_3P$	646	0.01	0.91
13	Irganox 1076	$C_{35}H_{62}O_3$	530	0.01	1.48
14	Tinuvin 144	$C_{42}H_{70}O_5N_2$	682	0.01	0.24
15	Irganox 245	$C_{34}H_{50}O_9$	602	0.01	0.44
16	Irganox 259	$C_{40}H_{62}O_6$	638	0.02	0.35
17	Irganox 1035	$C_{38}H_{58}O_6S$	642	0.01	0.58
18	Irganox 565	$C_{38}H_{56}ON_4S_2$	588	0.02	0.50
19	Crodamide	$C_{40}H_{76}O_2N$	588	0.01	0.66
20	Irganox 1098	$C_{40}H_{64}O_4N_2$	636	0.01	0.65
21	Irganox 3114	$C_{48}H_{69}O_6N_3$	783	0.02	0.68
22	Irganox 1330	$C_{54}H_{78}O_3$	774	0.01	0.29
23	Irganox PS802	$C_{42}H_{82}O_4S$	682	0.01	0.72
24	Irganox 1010	$C_{73}H_{108}O_{12}$	1176	0.01	1.50

times and quantitation. Average retention times and absolute peak areas for five runs are shown in Table 1. Relative standard deviations are within 0.04% for the retention times and 2% for the raw peak areas, even for the polar compounds such as erucamide and Irgaphos 168. This illustrates the robustness of HTCGC.

The combination of CGC with mass spectrometry (CGCMS) can also be used successfully for the analysis of these polymer additives. Helium was the carrier gas in the GCMS combination and this had some consequences on the selection of the column. The column length was reduced to 12 m, the i.d. to 0.2 mm and the film thickness to 0.11 μm. With helium at an inlet pressure of 10 kPa and operated in the constant flow mode, Irganox 1010 eluted at 39.5 min.

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Detectors

The use of the universal flame ionization detector (FID) or selective detectors such as nitrogen–phos-

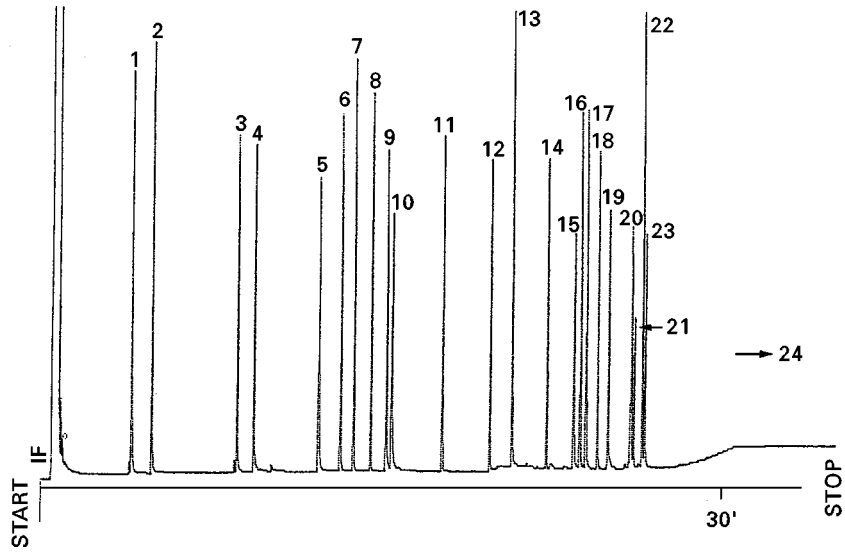

Figure 2 HTCGC analysis of the polymer additives using constant pressure mode. Peak identifications are given in Table 1.

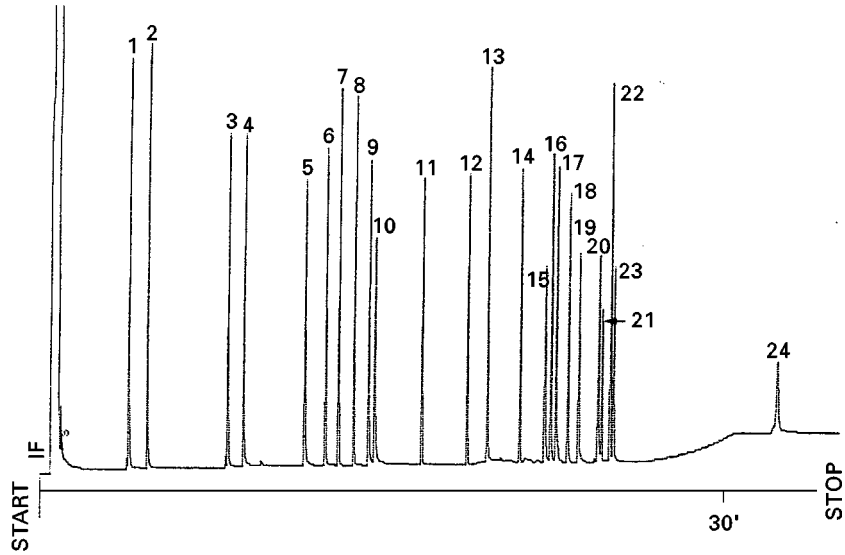

Figure 3 HTCGC analysis of the polymer additives using constant flow mode. Peak identifications are given in Table 1.

phorus detection (NPD) or electron-capture detection (ECD) does not pose any problem in HTCGC. Moreover, the spectroscopic techniques mass spectrometry (MS), atomic emission detection (AED) and inductively coupled plasma mass spectroscopy (ICPMS) are compatible with HTCGC. As an illustration, **Figure 4** shows the element-specific chromatograms for the phosphorus line at 178.1 nm (A) and the sulfur line at 181.4 nm (B) for the sample listed in Table 1 with the AED detector. The transfer line and cavity temperatures of the AED were set at 340°C. The other chromatographic conditions were very similar to those used for Figure 3.

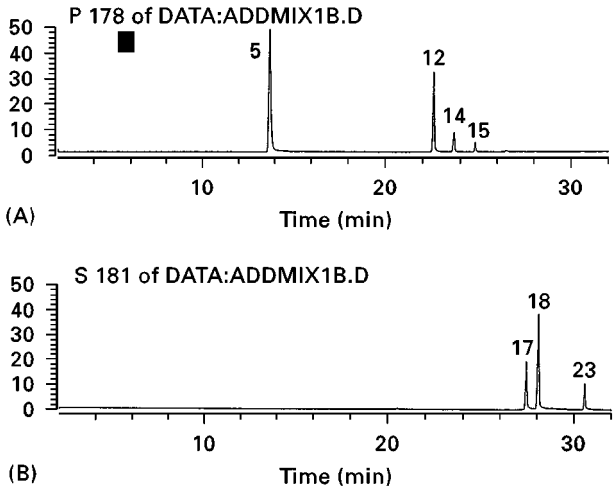

Figure 4 Element-specific chromatograms of the polymer additives recorded with atomic emission detection: (A) phosphorus line at 178.1 nm; (B) sulfur line at 181.4 nm.

Applications of HTCGC

In the framework of this contribution, it is impossible to review all applications of HTCGC. Some of the most relevant applications are detailed.

Hydrocarbons

HTCGC is nowadays intensively applied for the calculation of the true boiling point distribution of heavy petroleum products (simulated distillation or SIMDIS). In this type of analysis, high resolution is not wanted. The most desirable prerequisite the technique should fulfil is complete and quantitative elution. Hydrocarbons up to C_{130} elute quantitatively on short capillary columns coated with a thin film of methylsilicone.

Lipids

The qualitative and quantitative elucidation of glycerides is an important analysis in different fields, e.g. characterization of natural products, of food products, in lipid metabolism studies, bacterial identification, etc. A variety of techniques is routinely applied including liquid chromatography (LC), supercritical fluid chromatography (SFC) and HTCGC. Of these techniques, HTCGC provides the highest resolution in the shortest analysis time. The HTCGC analysis of a standard mixture of silylated mono-, di- and triglycerides is shown in **Figure 5**. The analysis was performed on a 12 m × 0.32 mm i.d. × 0.17 μm methylsilicone column programmed from 80°C to 380°C at 15°C min. Hydrogen was the carrier gas at 65 cm s^{-1} and both cool on-column and PTV injection could be applied.

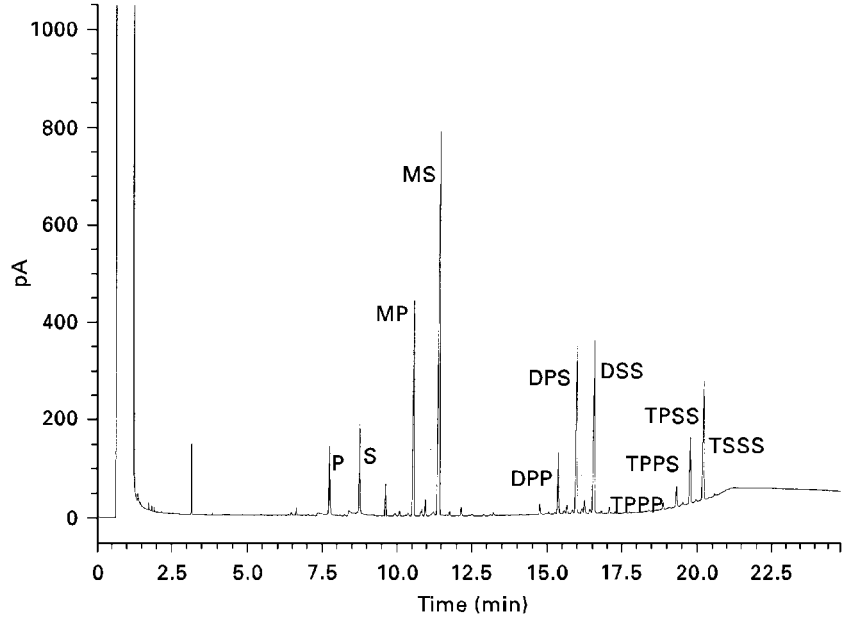

Figure 5 Analysis of silylated mono (M)-, di(D)- and tri(T)-glycerides. P, palmitic acid; S, stearic acid.

It is often claimed that HTCGC yields erratic quantitative results for triglycerides because of decomposition. Problems can indeed be encountered with oils containing large amounts of highly unsaturated triglycerides such as trilinolenin (LnLnLn), which tend to polymerize and not to decompose. For quantification of such lipids, calibration is necessary. Most of the oils and fats, however, can be analysed perfectly well by HTCGC. On apolar columns of the methylsilicone type, triglycerides are separated according to the carbon number. On polarizable diphenyl (50–65%) dimethylsilicone phases, besides a carbon number separation, lipids are also separated according to the different combinations of saturated and unsaturated fatty acids in the triglycerides. **Figure 6** shows the analysis in 3 min of the lipids in palm oil. The diglycerides with carbon numbers 32 to 36 and the triglycerides from C46 to C56 were separated on a 5 m × 0.25 mm i.d. × 0.1 μm methylsilicone in a temperature programmed run from 290°C to 350°C at 30°C min^{-1} applying cool on-column injection and with hydrogen as carrier gas at 103 kPa. **Figure 7** shows the analysis of inter-esterified cocoa butter on a poly(dimethyldiphenylsiloxene) column 25 m × 0.2 mm i.d. × 0.1 μm film. The sample was introduced via a PTV injector. Besides a carbon number separation, the triglycerides on this phase are also separated according to degree of unsaturation.

HTCGC is nowadays the method of choice in the chocolate industry to control the natural origin of cocoa butter and to elucidate the addition of cocoa butter equivalents and/or nut oils.

Detergents and Surfactants

Most of the non-ionic surfactants can be analysed by HTCGC. This is illustrated in **Figure 8** with the analysis of trimethylsilylated Triton X-100, an alkylphenol polyethoxylate, on a 10 m × 0.32 mm i.d. × 0.1 μm diphenyl (5%) dimethylsilicone column. The temperature was programmed from 65°C to 200°C at 40°C min^{-1} and then to 390°C at 8°C min^{-1}. Cool on-column injection was used. Helium was the carrier gas at 70 kPa. The relative standard deviation of retention times was lower than 0.1% and on raw peak areas lower than 0.7%. Another type of non-ionic surfactant is the polyethylene glycols (PEGs). HTCGC is able to analyse samples containing PEGs up to 1300 Da (PEG 1000).

Oligosaccharides

Oligosaccharides are thermally unstable and have to be derivatized into the well-known oxime-trimethylsilyl derivatives. The limits of HTCGC are illustrated in **Figure 9**. An oligosaccharide sample, obtained by hydrolysis of insulin extracted from *Cichorium intybus*, with DPs ranging from DP1 to DP12 could be analysed in approximately 30 min on a 10 m × 0.53 mm i.d. × 0.1 μm methylsilicone column. The degree of polymerization (DP) is the number of sugar units in the oligosaccharides. Both the pressure (50–200 kPa) and the temperature (100–430°C) were programmed to elute the high DP numbers. The analysis was performed on an aluminium-clad column. In this application resolution is sacrificed for speed of elution.

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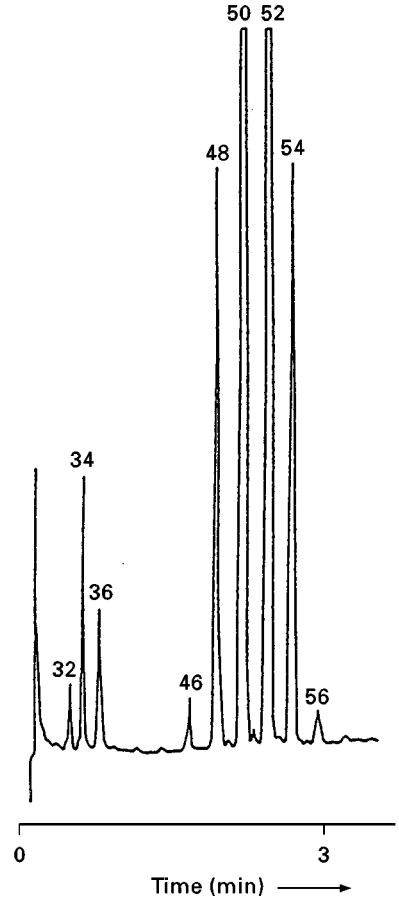

Figure 6 Fast carbon number separation of palm oil.

Emulsifiers

Organic substances added to food products to form emulsions are very complex mixtures. At present, there is no universal analytical method to elucidate the nature and origin of an emulsifier. Nevertheless,

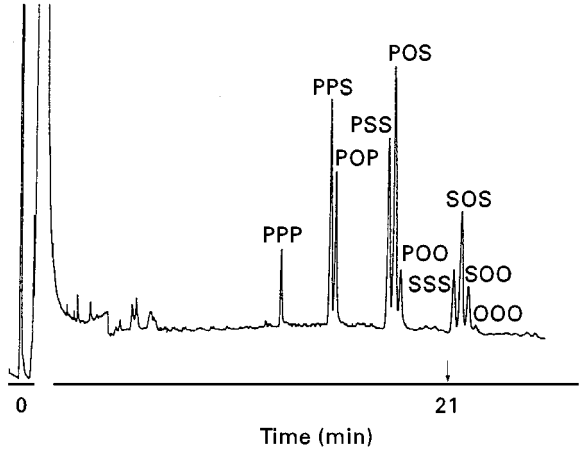

Figure 7 Analysis of inter-esterified cocoa butter. P, palmitic acid; S, stearic acid; O, oleic acid.

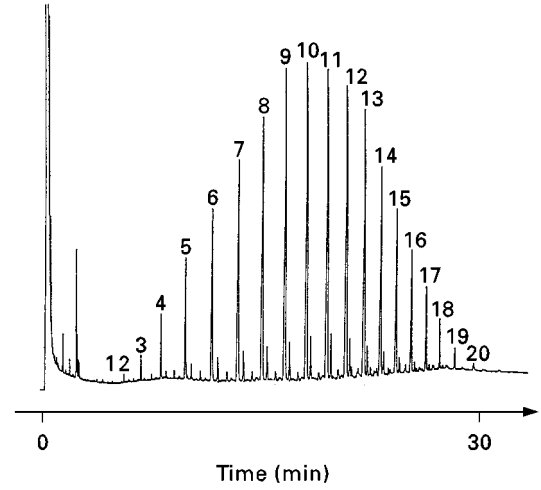

Figure 8 HTCGC analysis of Triton X-100 as trimethylsilyl derivatives. Numbering: ethylene oxide (EO) units.

HTCGC allows the characterization of a large number of emulsifying mixtures. This is illustrated with the analysis of two commercially available emulsifiers: one based on sorbitol (**Figure 10**A) and one on lactic acid (Figure 10B). Both samples were analysed on an automated HTCGC instrument (HP 6890) equipped with a $13\,m \times 0.32\,mm$ i.d. $\times 0.1\,\mu m$ methylsilicone column and a cool on-column injector. The silylated samples were injected in the oven track mode with an oven temperature programme from 70 to 370°C at 15°C min^{-1}. Hydrogen was the carrier gas at 25 kPa.

Miscellaneous

This overview of applications of HTCGC is far from complete and could be extended with the analysis of metal porphyrins in crude oils (geomarkers), of tall oil components, of mycolic acids, of antifoam agents, of antibiotics, etc. The applied methodologies are, however, similar to those described for the other applications.

Conclusion

HTCGC is a powerful analytical method for the analysis of high molecular weight compounds. Instrumentation and columns are commercially available. For some applications, derivatization into stable volatile substances is required.

In HTCGC we cannot expect spectacular new developments because the thermal stability of the compounds being separated is the limiting factor. The MW range can be expanded a little by applying high speed columns, i.e. short lengths and small internal diameters. By reducing the residence time in the column, the thermal stress is reduced as well.

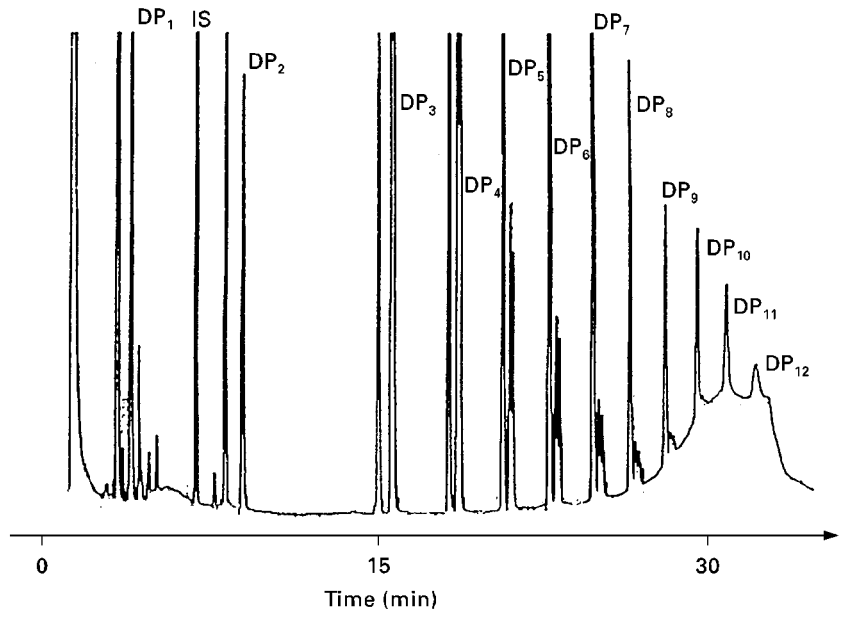

Figure 9 HTCGC analysis of an oligosaccharide sample using a temperature and pressure programme.

On the other hand, we will see more and more applications in the literature because CGC is always superior in terms of efficiency and speed of analysis compared to the other separation methods. With state-of-the-art HTCGC a number of applications presently carried out with LC or SFC, can be done much better with GC.

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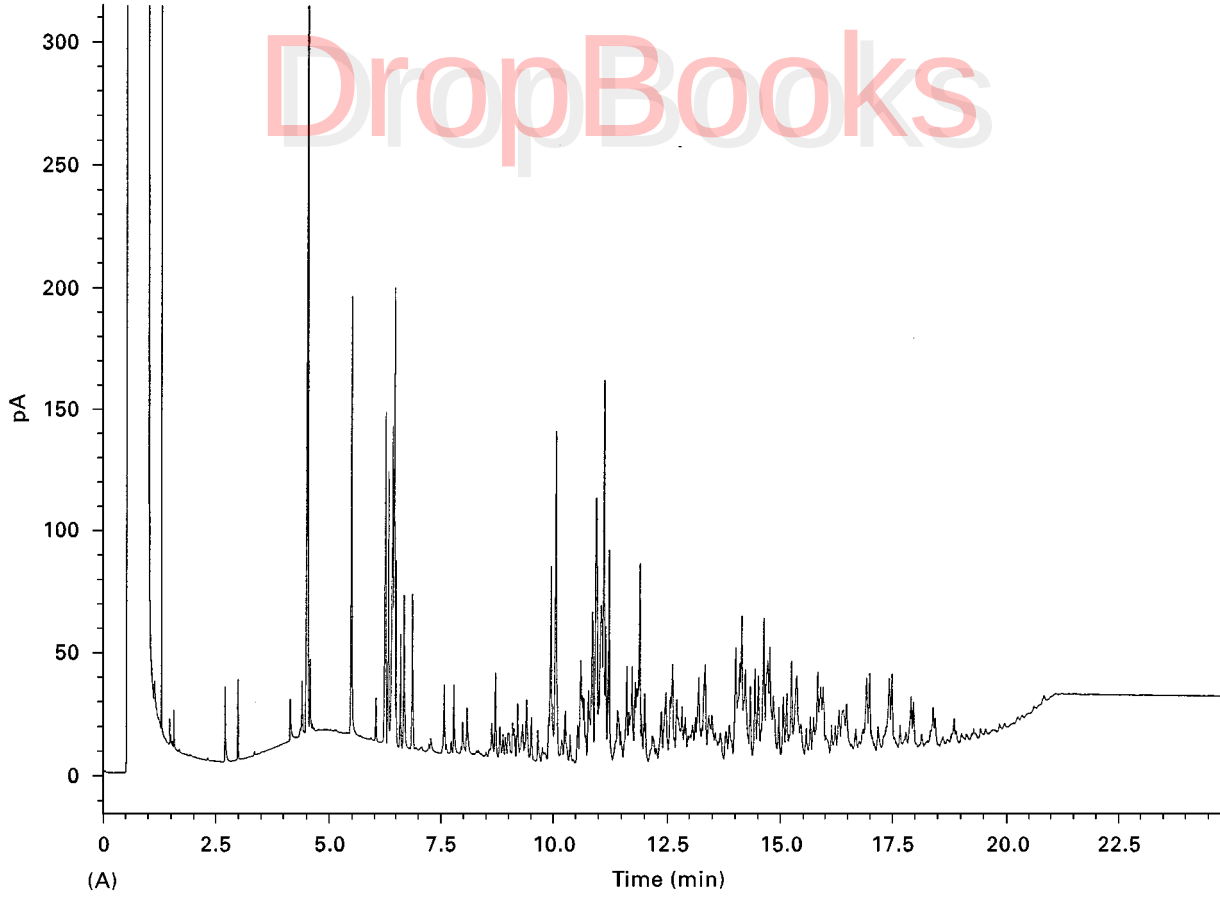

Figure 10 Analyses of emulsifiers based on sorbitol (A) and on lactic acid (B).

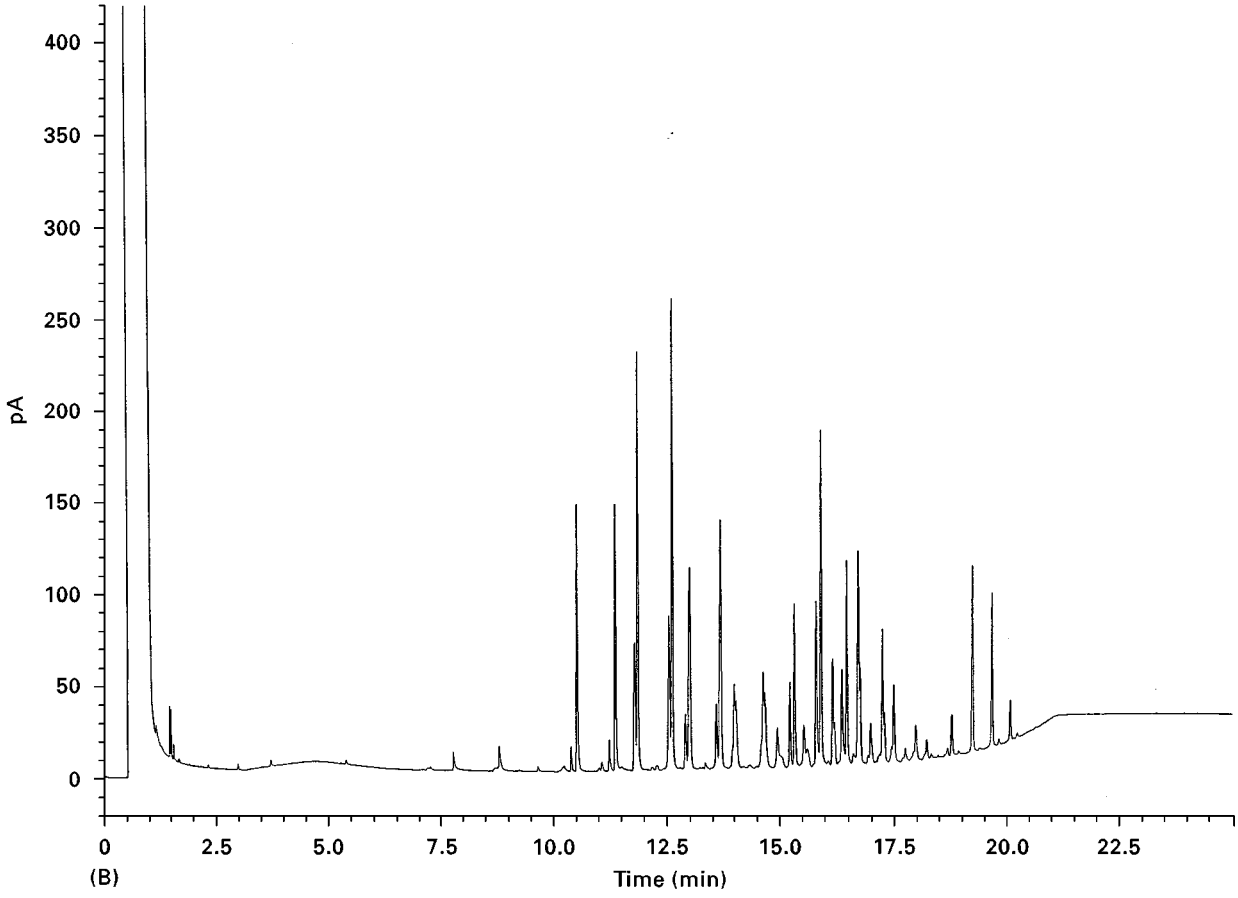

Figure 10 *Continued*

See also: **II/Chromatography:** Column Technology; Derivatization; Detectors: General (Flame Ionization Detectors and Thermal Conductivity Detectors); Detectors: Mass Spectrometry; Detectors: Selective; Sampling Systems. **III/Lipids:** Gas Chromatography. **Oils, Fats and Waxes:** Supercritical Fluid Chromatography. **Petroleum Products:** Gas Chromatography.

Further Reading

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